PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
01263/1		
International application No. PCT/IB2004/002993	International filing date *(day/month/year)* 13.09.2004	Priority date *(day/month/year)* 29.09.2003
International Patent Classification (IPC) or national classification and IPC C12P33/02, C12P33/16		
Applicant PHARMACIA & UPJOHN COMPANY et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.10.2004	01.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Fausti, S Telephone No. +49 89 2399-7389

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/002993

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-8 as originally filed

   Claims, Numbers

   1-11 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	-
	No:	Claims	1-11
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-11
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. DOCUMENTS and ABBREVIATIONS.

AD: androst-4-ene-3,17-dione;
ADD: androst-1,4-diene-3,17-dione;
DTL: dehydrotestolactone.

Reference is made to the following documents:

D1: DE 956952 C;
D2: Kondo E., *Kogyo Kagaku Zasshi* (1964), Vol. 67, No. 5, pages 724-727 (Chemical Abstract Accession No. 1964:487819);
D3: Capek A. et Al., *Folia microbiol.* (1960) Vol. 5, pages 251-255 (Chemical Abstract Accession No. 1961:18544);
D4: WO 03/064674 A;
D5: US 4124607;
D6: US 2823171;
D7: GB 732557.

1.1 D1 discloses the fermentative oxidation of steroids by means of *Fusarium solani* or *F. caucasicum* (see claim 1). In a specific embodiment, AD is converted to DTL in the presence of *F. caucasicum* at a substrate concentration of about 1 gr/lt (see example 4 and the attached Beilstein database entry).

1.2 D2 discloses the fermentative transformation of AD by *F. solani* involving the dehydrogenation of ring A, and the cleavage and lactonization of ring D (see the CA abstract).

1.3 D3 discloses the fermentative oxidation of progesterone by *F. solani*, *F. lateritium* and *F. caucasicum*. The intermediates of this fermentation process are AD and testosterone, with testolactone as a further metabolite. These intermediates and metabolites are further converted to the corresponding dehydrogenated derivatives (see the CA abstract).

1.3[a] In particular, D3 teaches that the final products are independent from the fermentation conditions and the composition of the fermentation medium (see the last sentence of the CA abstract).

1.4 D4 discloses the biosynthesis of ADD via the fermentative dehydrogenation of the ring A of AD by *Fusarium* spp., e.g. *F. solani* (see the abstract).

1.4[a] In a specific embodiment, the fermentation medium contains sunflower oil (see example 2).

1.5 D5 teaches that soybean oil and surfactants, like Triton X-100 (octylphenoxy polyethoxy ethanol), are among the additives commonly used in the preparation of sterol substrates for fermentation reaction (see the abstract and lines 5-10 on column 5).

1.6 D6 discloses the microbial conversion of steroids for the biosynthesis of DTL derivatives (see column 1, lines 19-25). In preferred embodiments, the converting microorganism is *F. solani*, which is fed with natural oils, like soybean oil (see: column 1, lines 26-36; paragraph joining columns 1 and 2).

1.7 D7 teaches that the fermentative oxidation of steroids can be carried out using natural oils (e.g. soybean oil) as the carbon source for the microorganisms. Such a carbon source is preferred because it enhances the availability of the steroid for conversion (see page 2, lines 54-73).

2. NOVELTY and INVENTIVE STEP.

2.1 The subject-matter of independent claim 1 is not novel over D1 because it discloses the fermentative conversion of AD into DTL by means of *F. caucasicum* (see point 1.1 above).

2.1[a] In addition, the subject-matter of claim 1 lacks novelty over D2 and D3 because these documents disclose fermentation processes, which are carried out in the presence of *Fusarium* spp., e.g.*F. solani* (see points 1.2 and 1.3 above). In these processes, DTL is inherently produced from AD in view of the fermentation reactions described in the documents.

2.2 Dependent claims 2-11 do not contain any features which, in combination with the

features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step, given the disclosures of the prior art cited in the Search Report.

2.2[a] In particular, the use of *F. solani* strains for the fermentative conversion of AD into DTL is anticipated in D2 and D3, and suggested by D1 and D6 (see points 1.1-1.3 and 1.6 above).

2.2[b] Higher substrate concentrations are obvious to the skilled person in view of the indication of D3 that the composition of the fermentation medium does not affect the final products of the reaction.

2.2[c] Two-step seed procedures and the addition of detergents and natural oils, like the octylphenoxy polyethoxyethanol "Triton X-100" and soybean oil, are within the customary practice followed by the skilled person (see for example points 1.5-1.7 above).

2.2[d] None of the dependent claims relates to a combination of features, which would accounts for the improved yield shown in the (very specific) examples of the present application. It is necessary that the effects, on the basis of which an inventive step could be acknowledged, are achieved over the whole claimed scope. This is clearly not the case of any of the present claims because, for example, the fermentation of AD by *F. solani* also produces ADD, thereby reducing the yield of DTL (see point 1.4 above).

3. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

3.1 Claims 1-11 relate to fermentation methods for the synthesis of compounds of pharmaceutical interest. These methods can be applied, for example, in the pharmaceutical industry, and are therefore to be considered industrially applicable according to article 33(4) PCT.